Rip11 influences the Axonal Transport of Rab7, but not Huntingtin.

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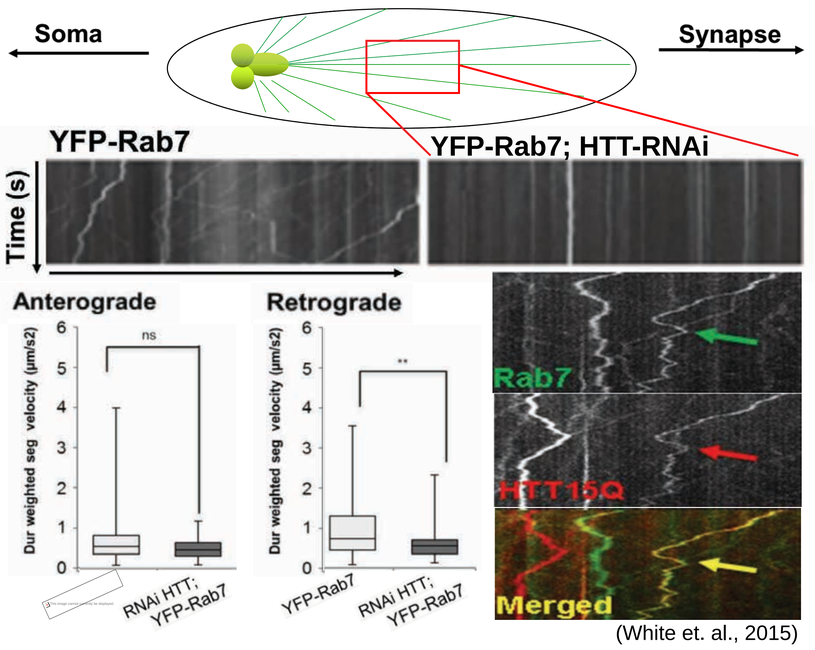
Abstract

Huntingtin (Htt) is the protein involved in Huntington's disease and is enriched in neurons. Htt acts as a scaffolding protein that associates with various proteins vital for neuronal function and viability. Recently, our lab demonstrated that reduction of Drosophila Htt selectively perturbed the retrograde motility of Rab7 containing vesicles. Htt and Rab7 also co-migrated together within axons (White et. al., 2015). Htt and Rabs have been shown to interact with molecular motors via associations with Htt or Rab associated proteins. Here we test the hypothesize that specific Rab associated proteins, Rip11, Nuf, and Nmo, are involved in the motility of Htt and/or Rab7.

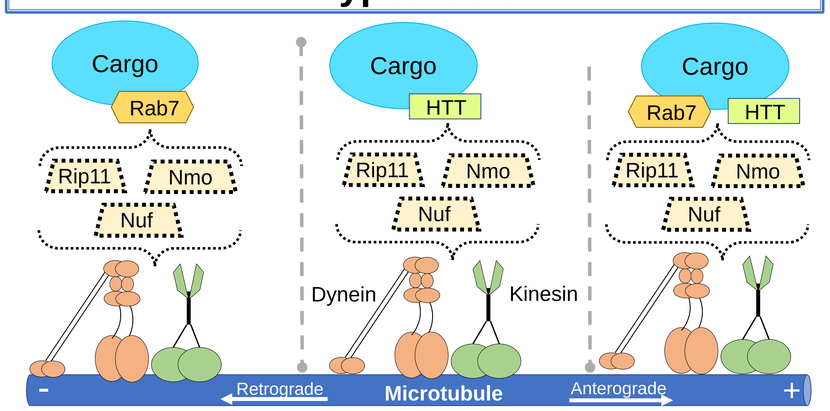
Evidence for a moving Htt-Rab7 complex



(White et. al., 2015)

White et al., 2015 demonstrated that Htt and Rab7 co-migrated, and that 70% reduction of dHtt perturbed retrograde motility of Rab7-containing cargo in larval nerves. Htt and Rab7 are thus functionally linked in a complex during axonal transport; however, other components of this cargo remain unknown. We hypothesize that Rip11, Nuf, or Nemo influence the axonal transport of Htt Rab7-containing cargo, due to their association with other Rabs. We will test this hypothesis using third instar drosophila larvae and we predict that our analysis will provide insight into the components of the moving Htt-Rab7 complex.

Hypothesis



Reduction of Nuf or Rip11 does not disrupt Huntingtin axonal transport

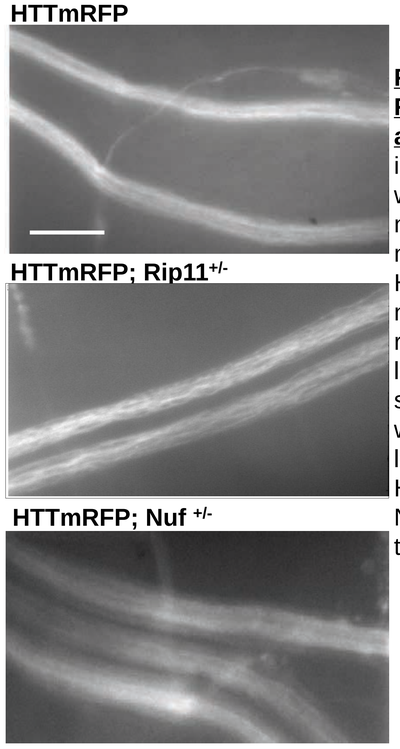


Figure 1: Reduction of Nuf or Rip11 does not disrupt HTT axonal transport. Drosophila third instar larvae expressing HTTmRFP were imaged at middle segmental nerves (60X) with epifluorescence microscopy. Larvae expressing only HTTmRFP show smooth staining in nerves. HTT-mRFP expression with reduction of Rip11 or Nuf, using loss-of-function mutations also had smooth staining in larval nerves without any axonal blockages. The lack of axonal accumulations of HTTmRFP indicates that Rip11 and Nuf do not disrupt Htt axonal transport.

n=3 For each genotype
calibration bar = 10 µm

Reduction of Rip11, but not Nuf , disrupts Rab7 axonal transport

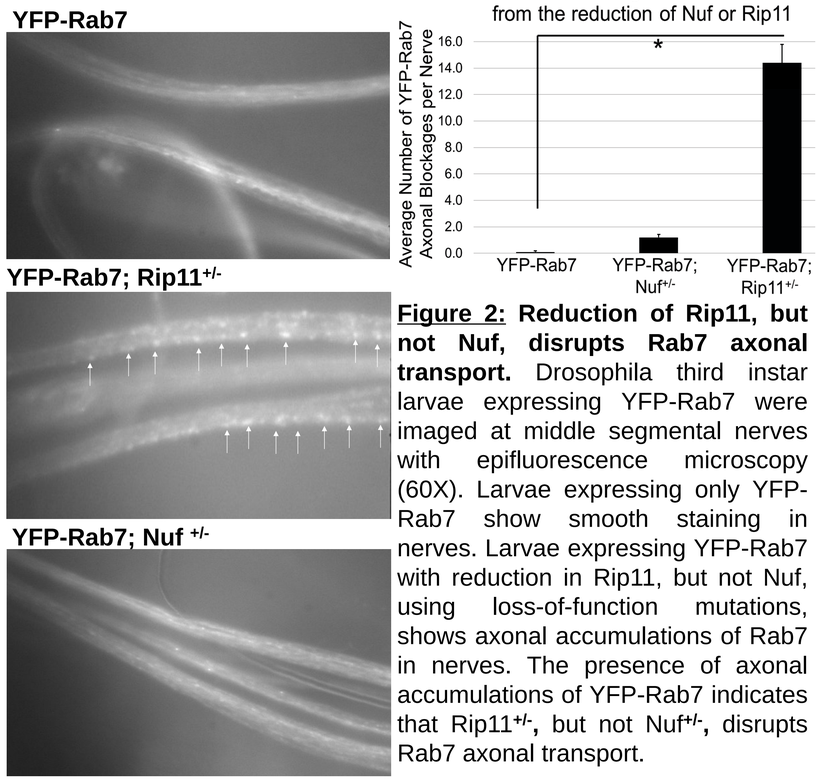
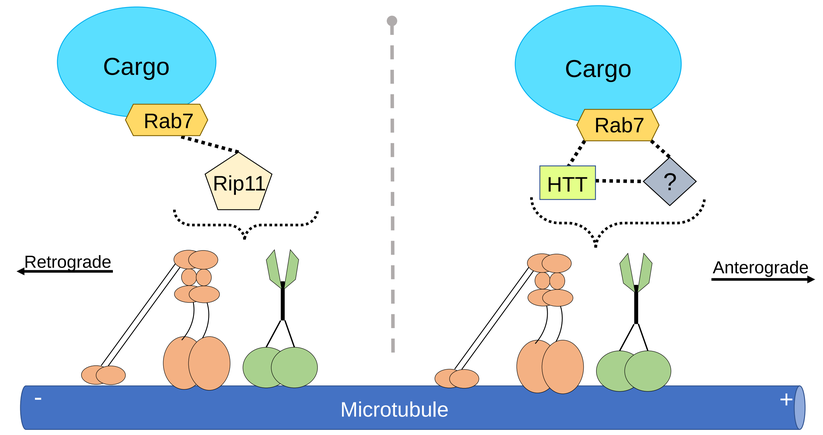


Figure 2: Reduction of Rip11, but not Nuf, disrupts Rab7 axonal transport. Drosophila third instar larvae expressing YFP-Rab7 were imaged at middle segmental nerves with epifluorescence microscopy (60X). Larvae expressing only YFP-Rab7 show smooth staining in nerves. Larvae expressing YFP-Rab7 with reduction in Rip11, but not Nuf, using loss-of-function mutations, shows axonal accumulations of Rab7 in nerves. The presence of axonal accumulations of YFP-Rab7 indicates that Rip11$^{+/-}$, but not Nuf$^{+/-}$, disrupts Rab7 axonal transport.

n=3 For each genotype
calibration bar = 10 µm

Working Model for Htt-Rab7 Cargo



LEFT: This working model represents Rab7-only vesicles associating with Rip11 during axonal transport. Although not intrical to all Rab7-containing vesicles, this work identifies a subset of Rab7-containing vesicles that require Rip11 for proper transport.

RIGHT: This working model represents Htt-Rab7 containing vesicles. Since neither Nuf nor Rip11 reduction disrupted Htt within the axon, both Nuf and Rip11 are unlikely to be with this vesicle. Future work will test other rab-associated proteins to identify associates with Htt-Rab7 vesicles.

Conclusions

1) Reduction of Nuf or Rip11 does not disrupt Htt motility.

2) Reduction of Rip11, but not Nuf, disrupts Rab7 motility
   - Rip11 causes a significant number of Rab7 axonal blocks in larval nerves

3) Neither Rip11 nor Nuf likely associate with the moving Htt-Rab7 complex.

Future Directions

- Are other Rab-associated proteins responsible for the motility of Htt-Rab7 vesicles?
- Are known Huntingtin-associate proteins responsible for the motility of Htt-Rab7 vesicles?
- What cargo does this Htt-Rab7 vesicle carry along the axon?

Acknowledgements

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